

I. CLAIM LISTING

1-15 Cancelled

16 (New) In a bioassay for the detection in human bodily fluids of a target carbohydrate antigen that is characteristic of a bacterium causative of human ear and respiratory tract infections, which bacterium also is known to colonize the nasopharyngeal area of children of an age up to about 12 years without causing infection, the improvement which reduces the incidence of results falsely indicative of the presence of infection in said children who are nasopharyngeally colonized but are otherwise healthy, which improvement maintains the sensitivity of said bioassay to the presence in bodily fluids of said carbohydrate antigen and also maintains the specificity of said bioassay at not less than 90%, and consists in reducing the total amount of antibodies to said carbohydrate antigen employed per test by an amount that is determined empirically in identical bioassay tests in which the amount of antibody present is varied and bioassay tests are run identically for each variation on samples of bodily fluid taken from both (1) otherwise healthy children known to be nasopharyngeally colonized by the bacterium of which the antigen is characteristic and (2) children known to have an ear or respiratory infection caused by the same bacterium.

17 (New) A bioassay according to Claim 16 wherein the target antigen is a carbohydrate antigen characteristic of a bacterium selected from among *Streptococcus pneumoniae*, nontypable *Haemophilus influenzae*, *Moraxella catarrhalis* and *Staphylococcus aureus*.

18 (New) A bioassay according to Claim 16 wherein the target antigen is the C-polysaccharide cell wall antigen common to all serotypes of *Streptococcus pneumoniae*.

19 (New) A bioassay according to Claim 16 conducted on an immunochromatographic (“ICT”) test strip upon which (a) tagged antibodies to the target carbohydrate antigen have been movably deposited near the sample introduction end of said strip, whereby liquid sample, upon introduction to the strip, picks up the movable deposit of said tagged antibodies and flows together therewith along the strip, thereby enabling formation of tagged antibody-antigen conjugates if target antigen is present in the sample, and (b) antibodies to the target antigen have been immovably deposited to form the capture line located near the end of said strip remote from its sample introduction end, so that tagged antibody-antigen conjugates in the flow stream of sample and tagged antibody react with said immovable antibodies to form tagged antibody-antigen-immovable antibody “sandwiches” upon contact, causing tag to mass upon the capture line.

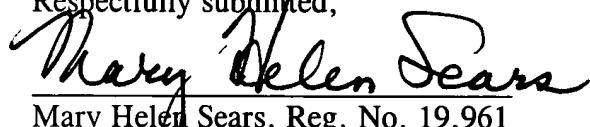
20 (New) A bioassay according to Claim 19 wherein the antibodies are antibodies to the C-polysaccharide antigen of *Streptococcus pneumoniae* which have been affinity purified, the tag material is colloidal gold and the total amount of antibodies needed per bioassay to reduce the incidence of results falsely indicative of the presence of infection in children who are nasopharyngeally colonized with *Streptococcus pneumoniae* but otherwise healthy has been determined in empirical tests, and is attained by (a) placing the movable deposit of tagged antibodies on the ICT strip by applying a solution of said antibodies having an optical density of 1.5 and (b) depositing the immovable antibodies constituting the capture line from a solution containing 0.3 mg./ml. of antibodies delivered to the strip at a rate of 0.5 ml. per 6 mm. of strip with the delivery tip of a

precision pump.

21 (New) In an ICT bioassay for the detection in human bodily fluids of a carbohydrate antigen that is characteristic of a bacterium causative of human ear and respiratory tract infections, which bacterium also frequently colonizes the nasopharyngeal area of children of an age up to about 12 years without causing infection, the improvement which reduces the incidence of test results falsely indicative of the presence of infection in said children who are nasopharyngeally colonized but are otherwise healthy, which improvement maintains the sensitivity of said bioassay to the presence in said bodily fluids of said carbohydrate antigen and also maintains the specificity of said bioassay at not less than 90% and consists in adding at least one immovable "scrub" line on the test strip, located just prior to the capture line in the sample flow path of the ICT test strip, for the purpose of removing excess target antigen in the sample by "scrubbing out" an identical portion of said target antigen from samples of bodily fluid obtained from both (a) otherwise healthy children who are nasopharyngeally colonized by the bacterium of which the target antigen is characteristic and (b) children who are infected by the same bacterium, wherein the number of capture lines, the concentration of antibody deposited on each capture line and the extent, if any, to which the concentration of antibodies otherwise employed in the test is modified in order to obtain the stated results have all been determined empirically in bioassay tests wherein each of (i) the number of "scrub" lines, (ii) the concentration of antibody on each "scrub" line, and (iii) the total amount of antibodies otherwise present, per test, were determined in identical bioassays representing each variation that were run identically

on samples of bodily fluid taken from each of (1) otherwise healthy children known to be nasopharyngeally colonized by the bacterium of which the target antigen is characteristic and (2) children known to have an ear or respiratory infection caused by said bacterium.

Respectfully submitted,



Mary Helen Sears

Mary Helen Sears, Reg. No. 19,961
Attorney for Applicants
The M.H. Sears Law Firm, Chartered
910 Seventeenth Street, N.W.
Washington, D.C. 20006
Telephone: 202-463-3892
Telecopy: 202-463-4852